

The University of Chicago

CYTOLOGICAL STUDIES IN THE
MYRICACEAE

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF BOTANY

1937

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By
JAMES STOKES

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CYTOLOGICAL STUDIES IN THE MYRICACEAE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 485

JAMES STOKES

(WITH FORTY FIGURES)

Introduction

According to SMALL (4) the genera *Myrica* and *Cerothamnus* are dioecious and *Comptonia* is monoecious. Partly for this reason the Myricaceae were selected for cytological study in order particularly to determine whether heterochromosomes are present. Inasmuch as such elements were not found, the meiotic processes in the microspore mother cells were studied in the following species: *Myrica cerifera* L., *M. pumila* Michx., *M. carolinensis* Mill., and *Comptonia peregrina* (L.) Coulter.

Plants of *M. cerifera*, *M. pumila*, and *M. carolinensis* were collected in the vicinity of Valdosta, Georgia. *M. pumila* is usually found on sandy acid pinelands. The distribution of *M. cerifera* overlaps that of *M. pumila*; it is common also in hammocks and swamps. *M. carolinensis* is restricted to low or wet acid pinelands, edges of hammocks, and stream banks.

Field observations on these species indicated that all are strictly dioecious. In order to check the field observations more carefully, representative specimens of *M. cerifera*, which were sufficiently mature to have produced strobili the previous season, were transplanted to the campus of the Georgia State Womans College. Although the new environment was somewhat drier than that of their native habitat, they grew luxuriantly.

Microscopic examination of staminate strobili of various ages gave no evidence of the presence of carpellate flowers. Although a majority of the carpellate strobili of a particular plant proved to be strictly carpellate, others were androgynous. In the latter the staminate flowers developed among the carpellate long after the disappearance of the staminate strobili of the strictly staminate plants. The anthers of such flowers were well developed and contained ap-

parently functional pollen. All variations observed were in the direction of carpellate to staminate. No perfect flowers were observed among either staminate or carpellate strobili. In all three species staminate strobili developed much earlier than the carpellate.

Specimens of *Comptonia peregrina* were located in the vicinity of Thornton, Illinois. Macroscopic examination indicated that the plants were monoecious. Microscopic examination of staminate strobili showed no carpellate flowers, but many of the carpellate strobili were androgynous. In such androgynous strobili staminate flowers appear among the carpellate during the late stages of seed development. In *Comptonia*, as in *Myrica*, the staminate strobili develop earlier than the carpellate.

Previous investigations of various species of *Myrica* have shown that variations in sexual expression are not infrequent. DAVEY and GIBSON (3) found that in *M. gale* there always exists a small proportion of monoecious plants exhibiting all gradations between strictly staminate and strictly pistillate. The series includes plants or shoots (a) bearing staminate and pistillate catkins of the normal type, (b) bearing androgynous catkins, (c) the bulk of the catkins consisting of hermaphroditic flowers. These writers state (3): "It has been found that the sex (if it may be so termed) of a bush or shoot may vary from year to year. The variations observed during several years have been almost entirely in the direction of change from the pistillate to the staminate condition; but in the present season (1916) several instances of the reverse change have been noted."

CHEVALIER (1) examined certain monoecious species of this family, notably *Myrica californica*, *M. conifera*, and *M. pubescens*. He reported variations in these forms similar to those reported by DAVEY and GIBSON for *M. gale*. The causal complex underlying variations in sexual expression has not been determined. CHEVALIER suggested nutrition as the factor controlling the expressing of sex in the monoecious forms examined. In view of the results cited, as well as of my own observations, strict dioecism is not to be expected among the Myricaceae.

The Myricaceae might afford excellent material for studies in the relation of environment to variations in sexual expression.

Material and methods

Staminate strobili were collected in the field. Before fixation they were cut into small pieces, and in some cases individual flowers were fixed. Flemming's strong, medium, and weaker solutions were used. The medium solution gave satisfactory results. In addition, a variety of other fixatives such as Carnoy's, La Cour's, and various modifications of Navashin's solution were utilized. Satisfactory fixations were obtained with Sax's modification of Navashin's solution.

Belling's iron-aceto-carmin method proved effective in the choice of materials to be fixed for studies of chromosome numbers, but proved ineffective as a quick method of obtaining counts, as the chromosomes failed to stain sufficiently to render them clearly visible. The most satisfactory staining results were obtained with Heidenhain's iron-alum haematoxylin stain.

Investigation

Myrica cerifera

EARLY PROPHASE.—The cells of the primary sporogenous tissue undergo division in all planes, ultimately giving rise to a cylindrical mass. After completion of these premeiotic divisions, the definitive pollen mother cells enter upon their long period of growth.

The resting microspore mother cells are polyhedral and are readily distinguishable from adjacent cells by the size of their nuclei, the affinity of the nucleoli for organic dyes, and the density of their cytoplasm. The cell wall is thin; the cytoplasm is rather dense and granular. The nucleus is almost circular in cross section. The nucleolus stains sharply, in contrast with the remaining apparently rather scanty chromatic material. The chromatic threads appear irregular in outline and exhibit thickenings where they intersect (fig. 1).

The first indication of the beginning of the leptotene is a gradual thickening of some of the chromatic fibers. The netlike structure of the resting nucleus is replaced by long, thin, delicate threads so intertwined that individual ones cannot be followed throughout their total length (fig. 2). The leptotene stage is of short duration and the chromatic threads are too delicate to allow detailed observations

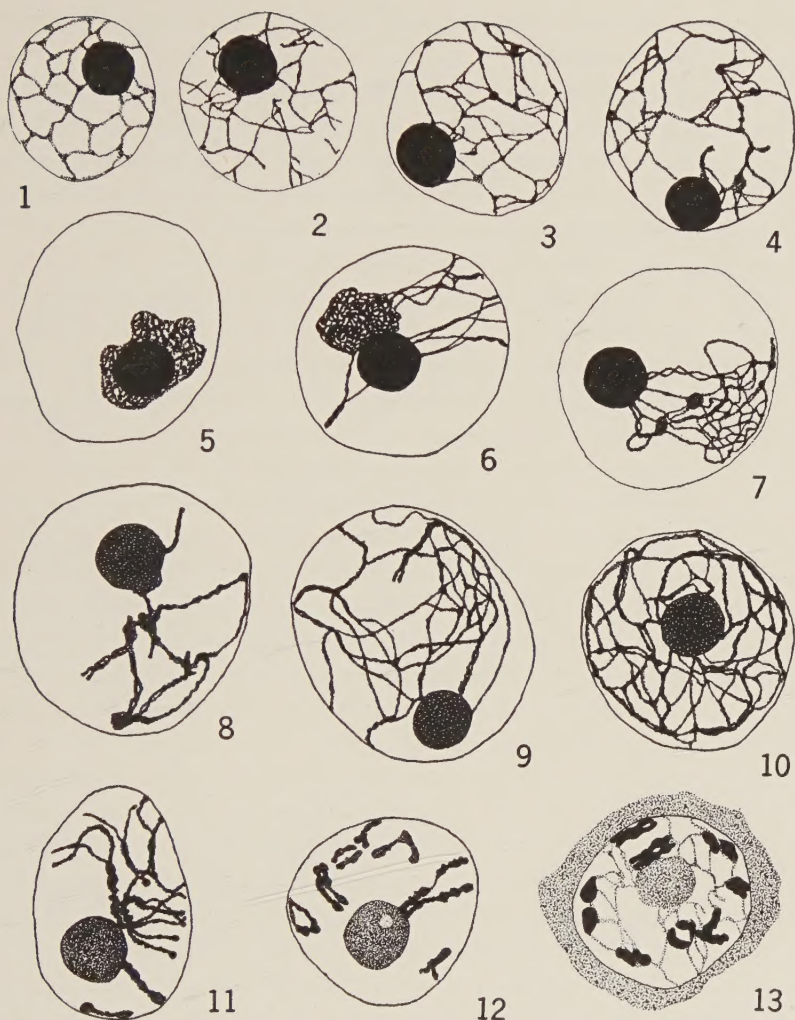
of their behavior. There is scanty evidence of pairing (fig. 3), but figure 4 may be interpreted as the initiation of the zygotene stage.

ZYGOTENE TO DIAKINESIS.—Soon the chromatic threads withdraw from the nuclear membrane and the nucleus passes into the condition of synizesis (fig. 5). Synizesis, whether an artifact or not, is of long duration. Extra nucleoli are not present at this or at subsequent stages of the first meiotic division. In most cases observed the synizetic knot is composed of an irregular mass of threads enveloping the nucleolus. All observations indicate that the nucleolus is never entirely free of the threads; at least one pair remains closely associated with this body, and possibly attached to it.

Evidence of the paired condition is more definite during the loosening of the synizetic knot (figs. 6, 7). The extension of loops or twists of the threads into the nuclear cavity is the first indication of the loosening of the knot, and apparently it occurs slowly. During early pachytene the bivalent threads lie irregularly distributed in the nucleus (figs. 8–10). They now become somewhat thicker, but no free ends are observable other than those which are due to cutting.

The transition from pachytene to diplotene is not readily observed in this material. The long threads characteristic of early pachytene undergo further shortening and thickening. The paired threads, which diverge widely at certain points, are held together by chiasmata (fig. 11). The doubleness of each chromosome of the bivalents, commonly observable in plant material with long chromosomes, was not seen in this material, which has very short chromosomes. The bivalent chromosomes now become progressively shorter and thicker and there is evident clumping and condensation of the chromatic materials as diakinesis is reached (fig. 12).

DIAKINESIS.—At diakinesis there is still no indication of the tetrad condition of the bivalents. The extreme shortening and thickening completely obscure the chromatids. In earliest stages of diakinesis the bivalents are still somewhat extended (fig. 12), but gradually the chromosomes of each pair approach each other very closely (fig. 13). The components of some bivalents are joined end to end, while others appear as V's and X's. At diakinesis the eight bivalents are peripherally placed in the nucleus. While this material, with very small and short chromosomes, is unsuitable for



FIGS. 1-13.*—*Myrica cerifera*: fig. 1, premeiotic nucleus of pollen mother cell; fig. 2, leptotene threads; fig. 3, early zygotene; fig. 4, pairing of threads in zygotene; fig. 5, synizesis; fig. 6, opening of synizetic knot; fig. 7, early pachytene; figs. 8-10, pachytene stages; fig. 11, diplotene stage with chiasmata; fig. 12, early diakinesis; fig. 13, late diakinesis showing eight bivalents.

* All figures drawn with 2 mm. apochromatic objective and 30X compensating ocular, at table level with the aid of a camera lucida.

a study of chiasma formation and behavior, figures 11-13 give evidence of their existence.

During the diakinetik stages the cytoplasm of the microspore mother cell has been undergoing changes. The peripheral region of the cytoplasm has become more vacuolate and a zone of granules of various sizes in the inner portion forms a definite perinuclear zone. The outer boundary of this zone is somewhat irregular in outline; its inner boundary adjoins the nuclear membrane (fig. 13).

The nucleolus, which during diakinesis has retained its affinity for organic dyes and has occupied a position at one side of the nucleus, gradually loses its staining capacity and begins to disappear. Nucleolar budding does not accompany the disorganization process. Shortly after the disappearance of the nucleolus the nuclear membrane becomes irregular in outline and begins to disintegrate. The nuclear cavity decreases in size, and the condensed bivalents pass from the peripheral region of the nucleus toward its center.

Spindle fibers appear in the nuclear cavity about and among the chromosomes. Some of these fibers appear to become attached to the bivalents at or near their ends (fig. 21) while others extend between the poles and remain unattached to the chromosomes. A typical multipolar diarch spindle is formed, and the bivalents, now closely grouped together, lie at the equator. In some cases the spindle appears pointed at the poles while in others it is rather broad.

FIRST METAPHASE TO END OF INTERKINESIS.—The bivalents are now arranged upon the equatorial plate (fig. 21). At this stage the chiasmata have become nearly or completely terminalized (figs. 20, 21). During the metaphase of the first meiotic division the chromosomes show no evidence of being composed of two chromatids (fig. 21). Gradually the homologous members of each bivalent move away from each other, the movement beginning at the spindle attachment regions. As a consequence of terminal spindle attachment and the probable resistance of chiasmata to the forces of disjunction, some of the chromosomes are drawn out into fine threads (fig. 20). The bivalents do not disjoin simultaneously. During the anaphase the two chromatids of each chromosome become visible (fig. 25) and in many of the dyads the two chromatids remain in contact



FIGS. 14-25.—Figs. 14-16, *Myrica carolinensis*: 14, 15, diakinetic stages; 16, late diakinesis showing eight bivalents. Figs. 17-19, *M. pumila*: 17, 18, diakinetic stages; 19, late diakinesis showing eight bivalents. Figs. 20-23, *M. cerifera*: 20, precocious disjunction of two bivalents at first metaphase; 21, cell with spindle figure showing relative size of spindle and cell; chromosomes in metaphase; 22, polar view of metaphase plate with eight bivalents; 23, early first anaphase. Fig. 24, *Comptonia peregrina*: spindle with chromosomes in early first anaphase. Fig. 25, *M. cerifera*: late first anaphase showing dyads.

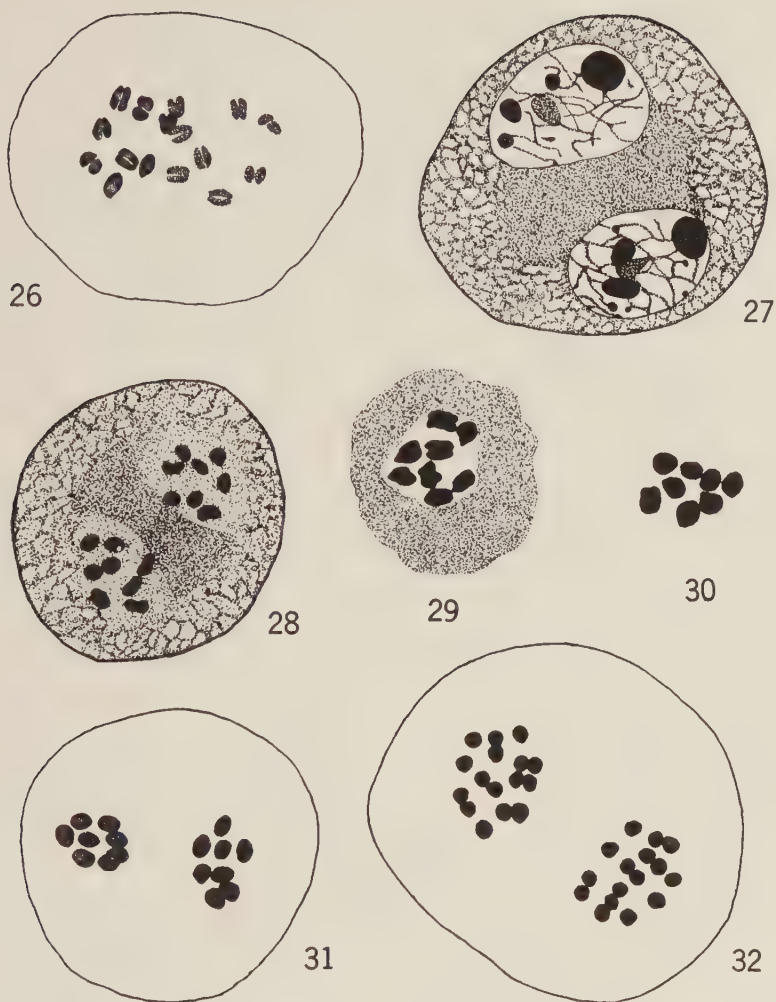
at the terminal attachment and diverge at the free ends, thus resulting in V-shaped figures (fig. 26). Upon reaching the poles the chromosomes usually mass together so closely that their individual identities cannot be distinguished. The aggregate of chromosomes later begins a loosening process and globular chromatic masses appear. Some of these bodies are nucleoli (fig. 27).

A nuclear membrane forms about each daughter nucleus and one or more nucleoli appear. During interkinesis the chromatic masses in the daughter nuclei continue the loosening process until the globular bodies disappear and the chromatic material is distributed on anastomosing threads. The spindle disappears with no indication of cell plate formation. The remains of the perinuclear zone are present between the daughter nuclei and contain numerous granules (fig. 27). At the termination of interkinesis small chromosomes appear in each daughter nucleus (fig. 28).

SUGIURA (5) reported the occurrence in *M. rubra* of an occasional constriction of the mother cell during interkinesis, but no incipient furrowing of the protoplast at interkinesis was observed in any of the species reported in this paper.

SECOND MEIOTIC DIVISION.—The spindles of the second meiotic division appear in the region previously occupied by the daughter nuclei, and are similar in appearance to those of the first division. The two spindles may lie parallel to each other (fig. 34) but more commonly they are at right or oblique angles. The surrounding cytoplasm contains many granules. As the chromosomes pass to the equatorial plates their double nature is evident (figs. 35, 36). Polar views of the four anaphase plates establish the haploid number as eight (fig. 37). After reaching the poles the chromosomes approach one another and form dense chromatic masses. About each polar group a nuclear membrane is formed (fig. 39). As the nuclei grow, the chromatic masses become loosened and anastomosing threads form. A nucleolus reappears in each nucleus. Continued loosening results in a resting nuclear condition characterized by a netlike arrangement of the threads (fig. 40).

The second division spindles persist until the formation of the nuclear membranes of the daughter nuclei and the region between the spindles is occupied with granules of variable sizes. Cell plates



FIGS. 26-32.—Figs. 26-28, *Myrica cerifera*: 26, first anaphase plates; 27, interkinesis; 28, polar view of second metaphase. Figs. 29, 30, *M. pumila*: 29, late diakinesis; 30, first metaphase plate. Fig. 31, *M. carolinensis*: second metaphase. Fig. 32, *Comptonia peregrina*: first anaphase plate showing secondary pairing of bivalents.

do not form. When the nuclei are completely reorganized they have withdrawn as far as possible from one another and lie close to the plasma membrane. A furrow begins in each depressed area of the mother cell and these furrows extend centripetally in such a manner that the tetranucleate protoplast is organized into four microspores, each with a single nucleus. The four young microspores are held together until the spores develop their own cell walls. After the appearance of a cell wall about each individual spore the mother cell wall begins to disintegrate, and with its disappearance the microspores are set free in the locules of the anther. Each young microspore is surrounded by a perinuclear zone of granules (fig. 40). The microspore grows rapidly and the nucleus develops a typical reticulum.

OTHER MYRICACEAE

Comparative studies of *M. cerifera*, *M. carolinensis*, *M. pumila*, and *Comptonia peregrina* indicate a close similarity in chromosome behavior. In all forms studied the meiotic divisions progress regularly.

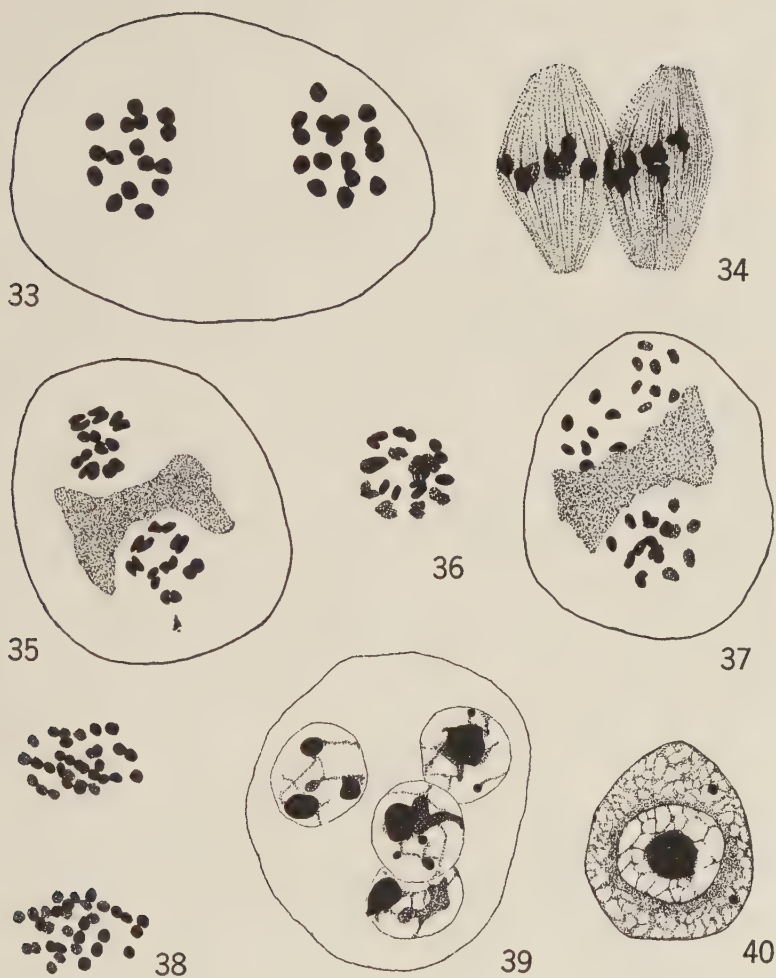
Myrica carolinensis

Examination of the meiotic processes in *M. carolinensis* reveals no outstanding differences from those described for *M. cerifera*. The same types of chromosome configurations are observable at diakinesis (figs. 14, 16). At diakinesis eight bivalents are evident and polar views of the first anaphase plate confirm this number (fig. 31). The chromosomes are characteristically short and thick and about as broad as long.

The plants used in this study and here referred to as *M. carolinensis* were thought originally to be a hybrid between *M. cerifera* and *M. carolinensis*. Meiotic studies showed no irregularities in chromosome behavior and no apparent disharmony between maternal and paternal chromosomes. This species is known to be variable in leaf size and pubescence.

Myrica pumila

The meiotic processes in *M. pumila* agree closely with those described for *M. cerifera* and for *M. carolinensis*. The same types of chromosome configuration described for *M. cerifera* are present at



FIGS. 33-40.—Fig. 33, *Comptonia peregrina*: first anaphase plate showing secondary pairing of bivalents. Figs. 34-37, *Myrica cerifera*: 34, spindles of second meiotic division; chromosomes in second metaphase; 35, 36, second metaphase stages; 37, second anaphase plates showing eight chromosomes in each plate. Fig. 38, *C. peregrina*: second anaphase plates; sixteen chromosomes in each plate, Figs. 39, 40, *M. cerifera*: 39 tetranucleate protoplast; 40, microspores.

diakinesis (figs. 17, 18). The number of bivalents is eight (fig. 19). At the first metaphase plate the eight bivalents show no recognizable differences in size (fig. 30).

Comptonia peregrina

Comptonia peregrina was formerly described as *Myrica asplenifolia* L. The general meiotic processes in *Comptonia* agree rather closely with those described for *M. cerifera*, differing mainly in the number of chromosomes. The bivalents pass to the equator and disjoin in an orderly manner (fig. 24). Lateral views of the first metaphases and polar views of the first anaphases establish the haploid chromosome number as sixteen. Polar views of the second anaphases also show sixteen (fig. 38).

Examination of the first metaphase, first anaphase, and second anaphases gives evidence of secondary pairing (figs. 32, 33). Different bivalents are closely associated but are not in contact. The relation of secondary pairing to the homology of the associated bivalents was recognized by DARLINGTON (2). On the evidences of secondary pairing and of chromosome number it seems probable that *Comptonia peregrina* is a tetraploid relative of the *Myrica* species studied.

Incidentally SUGIURA (5) found eight as the haploid number in *Myrica rubra* also.

Summary

1. The meiotic processes in the microspore mother cells of *Myrica cerifera* L., *M. pumila* Michx., *M. carolinensis* Mill., and *Comptonia peregrina* (L.) Coulter are described.

2. The Myricaceae range from polygamism through monoecism to dioecism. All variations observed were in the direction of changes from carpellate to staminate.

3. Material of *Myrica* and *Comptonia* was unsuitable for detailed studies on prophase stages, including chiasma formation. Synizesis is a constant feature in the species studied, whether an artifact or not.

4. Chiasmata become nearly or completely terminalized in late prophase.

5. At interkinesis the chromosomes are indistinguishable and cytokinesis does not occur at this stage.

6. Cytokinesis is by furrowing of the plasma membrane from the periphery, usually resulting in quadripartition with a tetrahedral arrangement of the microspores.

7. No evidence for the presence of heterochromosomes was observed in the meiotic divisions of any of the species studied.

8. *Comptonia peregrina* shows secondary pairing among its bivalents. This plant is probably a tetraploid relative of the *Myrica* species studied.

9. The haploid number of chromosomes is eight in *M. cerifera*, *M. pumila*, and in *M. carolinensis*, and sixteen in *Comptonia peregrina*.

The writer expresses his appreciation to Professor J. M. BEAL for the assistance given him during the course of this investigation, and to Professor C. E. ALLEN for suggesting the problem.

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